

PATENT ABSTRACTS OF JAPAN

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BOKU SEISHU**(54) METHOD FOR MANUFACTURING CONDROITIN SULFURIC ACIDS****(57)Abstract:**

PROBLEM TO BE SOLVED: To provide a method for manufacturing a condroitin sulfuric acid using a raw material which is easily available and by means of comparatively easy treatments.

SOLUTION: Fish scales are used as a raw material. They are subjected to a dissolving treatment, and then are subjected to a polypeptide removing treatment and a fractionation treatment, in this order, so as to obtain a condroitin sulfuric acid. For example, the scale dissolving treatment is carried out by allowing a protease to act on the scales in water. Next, a polypeptide formed as a by-product is removed from the solution thus formed. Then a condroitin sulfuric acid is obtained by fractional precipitation.

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CLAIMS

[Claim(s)]

[Claim 1] UROKO of fishes -- a raw material -- carrying out -- this -- solubilization processing, polypeptide clearance processing, and judgment processing -- one by one -- ***** -- the manufacture approach of the chondroitin sulfate characterized by things.

[Claim 2] The manufacture approach of the chondroitin sulfate according to claim 1 which acquires chondroitin sulfate according to fractional precipitation after removing a byproduction polypeptide from the solubilization liquid obtained by making a protease act on UROKO of fishes, solubilizing UROKO underwater, and then doing in this way.

[Claim 3] The manufacture approach of chondroitin sulfate according to claim 1 to 2 that chondroitin sulfate is chondroitin sulfate A and chondroitin sulfate C.

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DETAILED DESCRIPTION

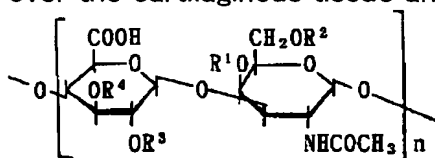
[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the approach of manufacturing chondroitin sulfate useful as eye lotions or a remedy of neuralgia and the arthralgia especially chondroitin sulfate A, and chondroitin sulfate C, by using UROKO of fishes as a raw material.

[0002]

[Description of the Prior Art] Chondroitin sulfate is one sort of the typical glycosaminoglycan widely distributed over the cartilaginous tissue and connective tissue of an animal, and is a general formula [** 1].



D-グルクロン酸

N-アセチル
D-ガラクトサミン

It has the structure with which it came out and 40-100 main repeat units combined the disaccharide expressed. With the number and joint location of a sulfuric-acid radical chondroitin sulfate A (a chondroitin-4-sulfuric acid and $R_1=SO_3H$ —) $R_2-R_4=H$ and chondroitin sulfate C (a chondroitin-6-sulfuric acid —) $R_2=SO_3H$, R_1 and R_3 , $R_4=H$, and chondroitin sulfate D (R_2 and $R_3=SO_3H$ —) R_1 , $R_4=H$, and chondroitin sulfate E (R_1 and $R_2=SO_3H$ —) It is divided into R_3 , $R_4=H$, chondroitin sulfate K (R_1 , $R_4=SO_4H$, R_2 , $R_3=H$), and chondroitin sulfate B (5-epimerization of many of dermatan sulfate and D-glucuronic acid is done, it serves as L-iduronic acid, and the R_1 has become a sulfuric-acid radical).

[0003] the chondroitin sulfate A in these chondroitin sulfate — mainly — the notochord of a sturgeon, and the cartilages nasi of a whale — moreover, chondroitin sulfate C — mainly — a shark — although the cartilage was manufactured as a raw material, respectively, since acquisition of a raw material needed complicated processing for a difficult top, mass production method was not completed and it did not escape becoming cost high.

[0004]

[Problem(s) to be Solved by the Invention] This invention is made using the raw material which is easy to come to hand for the purpose of manufacturing chondroitin sulfate by the easy processing in comparison.

[0005]

[Means for Solving the Problem] As a result of examining many things about the manufacture raw material of chondroitin sulfate, chondroitin sulfate A and chondroitin sulfate C are contained in UROKO of the fishes conventionally discarded while it had been unused, and this invention persons came to make this invention for the ability of these to be taken out comparatively easily based on a header and this knowledge.

[0006] The manufacture approach of the chondroitin sulfate characterized by things is offered, and this approach is underwater. namely, this invention — UROKO of fishes — a raw material — carrying out — this — solubilization processing, polypeptide clearance processing, and judgment processing — one by one — ***** — A protease is made to act on UROKO of fishes and UROKO is solubilized, and it consists of acquiring chondroitin sulfate according to fractional precipitation, after removing a byproduction polypeptide from the solubilization liquid obtained by next doing in this way.

[0007]

[Embodiment of the Invention] UROKO used in this invention approach may be which UROKO of freshwater fish and a saltwater fish. As UROKO of freshwater fish, UROKO, such as a carp, a crucian carp, a mass, and a goldfish, can be used, for example, and UROKO, such as Thailand, Suzuki, a salmon, a herring, and ETSU, can be used as UROKO of a saltwater fish, for example. Although it can be used only rinsing and removing dirt, if these UROKO is required, what was beforehand heated for 5 - 30 minutes at the temperature of 120-130 degrees C can also be used for it.

[0008] The solubilization processing in this invention approach adds and homogenizes the thin water solution of calcium acetate, and the water solution preferably buffered with the tris hydrochloric acid to UROKO of a raw material, is used as suspension, and is performed by making a protease add and react to this. This reaction is usually completed in the temperature of 30-40 degrees C in 5 - 40 hours. By this reaction, the protein part combined with chondroitin sulfate in UROKO dissociates, the byproduction of the polypeptide is carried out, with chondroitin sulfate, it dissolves in water and this becomes a water solution.

[0009] Thus, the obtained water solution is ** given to polypeptide clearance processing after filtration or centrifugal separation subsequently removes insoluble matter. This is performed by contacting the water solution except the above-mentioned insoluble matter on cation mold ion exchange resin. Under the present circumstances, it can precede contacting cation mold ion exchange resin, a water solution can be condensed if needed, and it can also dialyze to distilled water.

[0010] Thus, although the water solution containing chondroitin sulfate is obtained, in order to classify the object product from this water solution, by adding ethyl alcohol and raising concentration gradually into this water solution, chondroitin sulfate is settled and uptake is carried out for every fraction.

[0011] Thus, the chondroitin sulfate obtained from each fraction was identified by hydrolyzing with a digestive enzyme and detecting the saccharide to generate, making it contrast with the preparation of the origin which should be trusted. It hydrolyzes each sample using a chondroitinase, and this identification develops the obtained liquid by the thin film chromatography (it omits Following TLC), and the generated partial saturation disaccharide is made to color using a diphenylamine reagent, and it is performed by checking whether it is in agreement with the band of a preparation processed similarly. Thus, it was checked that the products of this invention approach are chondroitin sulfate A and chondroitin sulfate C.

[0012]

[Example] Next, an example explains this invention to a detail further.

[0013] solubilization of example 1 (1) UROKO -- a carp -- after rinsing UROKO 60g well, it heat-treated for 20 minutes at 120 degrees C. subsequently, UROKO heat-treated to 300ml (pH7.8) of 0.05M-tris hydrochloric-acid buffer solutions containing 0.02M-calcium acetate, and AKUCHINAZE E(Kaken Pharmaceutical make)300mg -- in addition, it homogenized with the homogenizer and suspension was prepared. Next, this suspension was made to react for three days at 37 degrees C, and UROKO was solubilized thoroughly. Thus, after having heated the obtained reaction mixture for 5 minutes at 100 degrees C, having carried out deactivation of AKUCHINAZE E, carrying out at-long-intervals alignment separation for 40 minutes and removing an insoluble element (20,000rpm), centrifugal supernatant liquid was condensed and one nights of concentrates were dialyzed to distilled water. It freeze-dried, after removing the polypeptide which separated at the above-mentioned reaction through the liquid which carried out dialysis processing in Dowex50-x8 (H+ mold) column (50x50mm) and neutralizing effluent by the sodium hydroxide. Thus, 330mg of freeze-drying objects was obtained.

[0014] (2) After dissolving 330mg of freeze-drying objects obtained by the fractional precipitation (1) of a meltable ghost in 50ml of 0.5M-sodium acetate, the acetic acid adjusted to pH4.5, ethanol was poured into this, by raising ethanol concentration gradually, chondroitin sulfate was settled and uptake was carried out as a fraction for every ethanol concentration. After adding 2 N-HCl of an amount (w/v) to each obtained fraction 1,000 times and hydrolyzing in 100 degrees C for 20 hours, each sugar composition was analyzed using the DX-500 sugar analysis apparatus (product made from die ONEKUSU). The result is shown in a table 1.

[0015]

[A table 1]

エタノール 画分 (%)	沈殿物収量 (m g)	糖組成 (μ g / m g)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0~40	5.0	11.15	43.54	13.38	47.46	18.23	2.69	0.00
40~50	49.0	11.67	28.23	129.41	157.37	29.62	1.18	0.00
50~60	4.0	14.01	33.75	32.50	64.74	27.76	3.49	0.00
60~70	6.0	17.50	69.02	6.79	60.09	35.09	41.52	12.22
70~	70.0	3.43	20.47	0.00	7.33	13.49	39.53	15.63

[0016] In addition, the code of the sugar in a table has following semantics.

Fuc : fructose Gal : Galactose GlcUA : Glucuronic-acid GalNAc: N-acetyl-galactosamine GlcNAc: N-acetylglucosamine NeuAc : N-acetylneuraminic acid NeuGc : N-glycolyl neuraminic acid [0017] As shown in this table, other glucuronic acid and N-acetyl galactosamine of a lot of than a fraction are contained in an ethanol fraction 40 to 50%, and the mole ratio of GlcUA and GalNAc is about 1:1. Moreover, the iduronic acid which is the constituent of chondroitin sulfate B was detected from neither of the fractions.

[0018] (3) An identification trial Every [of a chondroitin-sulfate-C preparation / preparation / which were obtained by (2) / 40 - 50% ethanol fraction 75microg and the chondroitin-sulfate-A preparation [it is written as the Seikagaku make speed signal generator grade, and Following ChsA] / [it is written as the Seikagaku make, speed signal generator grade, and Following ChsC] / 50micro / g] It dissolved in 100micro (pH5.0) of 0.025M sodium acetate buffer solutions I, respectively, and chondroitinase ASURO II[Seikagaku0.02] unit was made to melt and react to the 2micro of the same buffer solutions I as the above at 37 degrees C in addition for 20 hours. Subsequently, ethanol 300microl was added to reaction mixture, deactivation of the enzyme was carried out, and centrifugal separation removed insoluble matter.

[0019] It analyzed after condensing the obtained centrifugal supernatant liquid using the silica gel 60TLC plate (Merck Co. make). This result is shown in drawing 1 . As a developing solution of TLC in this case, 1-butanol / acetic acid / distilled water (capacity 2/1/1) was used, and the diphenylamine reagent was used for coloring of the partial saturation disaccharide generated by the reaction of chondroitinase ASURO II. Two partial saturation disaccharide bands were detected from the ethanol fraction 40 to 50%, and those mobility was in agreement with the band of two partial saturation disaccharides generated from ChsA and ChsC which were used as a preparation, respectively so that this drawing 1 might show.

[0020] the carp in example 2 example 1 (1) -- red sea bream UROKO 142g was used instead of UROKO 60g, it processed like the example 1, and 95mg of ethanol precipitate was obtained. The sugar composition for every ethanol fraction of this thing was analyzed like the example 1 (2). The result is shown in a table 2.

[0021]

[A table 2]

エタノール 画分 (%)	沈殿物収量 (m g)	糖組成 (μ g / m g)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0~40	6.1	5.30	167.44	26.53	181.04	29.13	23.07	0.00
40~50	23.3	2.40	102.91	197.65	264.73	83.68	11.25	7.85
50~60	11.7	0.44	53.58	25.50	57.11	33.75	58.51	55.34
60~70	5.2	4.10	4.77	6.66	93.67	0.00	99.41	29.32
70~	48.7	9.34	7.00	0.00	226.40	0.00	153.95	0.00

[0022] the carp in example 3 example 1 (1) -- ETSUUROKO 26g was used instead of UROKO 60g, it processed like the example 1, and 20.8mg of ethanol precipitate was obtained. The sugar composition for every ethanol fraction of this thing was analyzed like the example 1 (2). The result is shown in a table 3.

[0023]

[A table 3]

エタノール 画分 (%)	沈殿物収量 (mg)	糖組成 ($\mu\text{g}/\text{mg}$)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0～50	2.4	19.40	22.13	87.23	94.63	0.00	24.49	0.00
50～60	3.4	18.03	6.74	2.73	82.91	0.00	44.60	0.00
60～70	2.6	23.07	6.75	3.00	65.98	0.00	23.46	9.72
70～	12.4	12.93	8.40	1.55	138.72	0.00	113.39	25.76

[0024] the carp in example 4 example 1 (1) -- Lateolabrax UROKO 23g was used instead of UROKO 60g, it processed like the example 1, and 11.0mg of ethanol precipitate was obtained. The sugar composition for every ethanol fraction of this thing was analyzed like the example 1 (2). The result is shown in a table 4.

[0025]

[A table 4]

エタノール 画分 (%)	沈殿物収量 (mg)	糖組成 ($\mu\text{g}/\text{mg}$)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0～50	1.4	4.91	11.01	67.92	82.36	0.00	11.57	0.00
50～60	1.7	1.93	131.13	2.63	14.25	0.00	15.03	0.00
60～70	1.2	6.19	8.14	0.00	27.09	0.00	18.95	0.00
70～	6.7	7.62	4.66	0.00	175.68	0.00	112.81	12.59

[0026] Although chondroitin sulfate A and chondroitin sulfate C are contained in the protease processing object of fishes UROKO as shown in a table 2 thru/or 4, chondroitin sulfate B is not contained.

[0027]

[Effect of the Invention] According to this invention, the high chondroitin sulfate A and the chondroitin sulfate C of utility value can be manufactured by easy actuation as a remedy by using as a raw material UROKO of the fishes which are one sort of fishery trash.

[Translation done.]

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TECHNICAL FIELD

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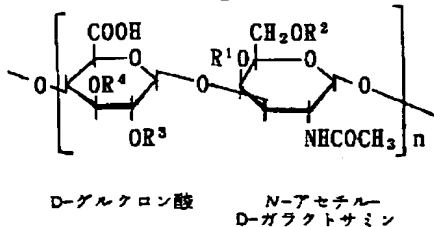
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PRIOR ART

[Description of the Prior Art] Chondroitin sulfate is one sort of the typical glycosaminoglycan widely distributed over the cartilaginous tissue and connective tissue of an animal, and is a general formula [** 1].



It has the structure with which it came out and 40-100 main repeat units combined the disaccharide expressed. With the number and joint location of a sulfuric-acid radical chondroitin sulfate A (a chondroitin-4-sulfuric acid and $R_1=SO_3H$ →) $R_2-R_4=H$ and chondroitin sulfate C (a chondroitin-6-sulfuric acid →) $R_2=SO_3H$, R_1 and R_3 , $R_4=H$, and chondroitin sulfate D (R_2 and $R_3=SO_3H$ →) R_1 , $R_4=H$, and chondroitin sulfate E (R_1 and $R_2=SO_3H$ →) It is divided into R_3 , $R_4=H$, chondroitin sulfate K (R_1 , $R_4=SO_4H$, R_2 , $R_3=H$), and chondroitin sulfate B (5-epimerization of many of dermatan sulfate and D-glucuronic acid is done, it serves as L-iduronic acid, and the R_1 has become a sulfuric-acid radical).

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EFFECT OF THE INVENTION

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MEANS

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[0007]

[Embodiment of the Invention] UROKO used in this invention approach may be which UROKO of freshwater fish and a saltwater fish. As UROKO of freshwater fish, UROKO, such as a carp, a crucian carp, a mass, and a goldfish, can be used, for example, and UROKO, such as Thailand, Suzuki, a salmon, a herring, and ETSU, can be used as UROKO of a saltwater fish, for example. Although it can be used only rinsing and removing dirt, if these UROKO is required, what was beforehand heated for 5 - 30 minutes at the temperature of 120-130 degrees C can also be used for it.

[0008] The solubilization processing in this invention approach adds and homogenizes the thin water solution of calcium acetate, and the water solution preferably buffered with the tris hydrochloric acid to UROKO of a raw material, is used as suspension, and is performed by making a protease add and react to this. This reaction is usually completed in the temperature of 30-40 degrees C in 5 - 40 hours. By this reaction, the protein part combined with chondroitin sulfate in UROKO dissociates, the byproduction of the polypeptide is carried out, with chondroitin sulfate, it dissolves in water and this becomes a water solution.

[0009] Thus, the obtained water solution is ** given to polypeptide clearance processing after filtration or centrifugal separation subsequently removes insoluble matter. This is performed by contacting the water solution except the above-mentioned insoluble matter on cation mold ion exchange resin. Under the present circumstances, it can precede contacting cation mold ion exchange resin, a water solution can be condensed if needed, and it can also dialyze to distilled water.

[0010] Thus, although the water solution containing chondroitin sulfate is obtained, in order to classify the object product from this water solution, by adding ethyl alcohol and raising concentration gradually into this water solution, chondroitin sulfate is settled and uptake is carried out for every fraction.

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EXAMPLE

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[A table 1]

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40~50	49.0	11.67	28.23	129.41	157.37	29.62	1.18	0.00
50~60	4.0	14.01	33.75	32.50	64.74	27.76	3.49	0.00
60~70	6.0	17.50	69.02	6.79	60.09	35.09	41.52	12.22
70~	70.0	3.43	20.47	0.00	7.33	13.49	39.53	15.63

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out, and centrifugal separation removed insoluble matter.

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[0021]

[A table 2]

エタノール 画分 (%)	沈殿物収量 (mg)	糖組成 (μg/mg)						
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40~50	23.3	2.40	102.91	197.65	264.73	83.68	11.25	7.85
50~60	11.7	0.44	53.58	25.50	57.11	33.75	58.51	55.34
60~70	5.2	4.10	4.77	6.66	93.67	0.00	99.41	29.32
70~	48.7	9.34	7.00	0.00	226.40	0.00	153.95	0.00

[0022] the carp in example 3 example 1 (1) — ETSUUROKO 26g was used instead of UROKO 60g, it processed like the example 1, and 20.8mg of ethanol precipitate was obtained. The sugar composition for every ethanol fraction of this thing was analyzed like the example 1 (2). The result is shown in a table 3.

[0023]

[A table 3]

エタノール 画分 (%)	沈殿物収量 (mg)	糖組成 (μg/mg)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0~50	2.4	19.40	22.13	87.23	94.63	0.00	24.49	0.00
50~60	3.4	18.03	6.74	2.73	82.91	0.00	44.60	0.00
60~70	2.6	23.07	6.75	3.00	65.98	0.00	23.46	9.72
70~	12.4	12.93	8.40	1.55	138.72	0.00	113.39	25.76

[0024] the carp in example 4 example 1 (1) — Lateolabrax UROKO 23g was used instead of UROKO 60g, it processed like the example 1, and 11.0mg of ethanol precipitate was obtained. The sugar composition for every ethanol fraction of this thing was analyzed like the example 1 (2). The result is shown in a table 4.

[0025]

[A table 4]

エタノール 画分 (%)	沈殿物収量 (mg)	糖組成 (μg/mg)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0~50	1.4	4.91	11.01	67.92	82.36	0.00	11.57	0.00
50~60	1.7	1.93	131.13	2.63	14.25	0.00	15.03	0.00
60~70	1.2	6.19	8.14	0.00	27.09	0.00	18.95	0.00
70~	6.7	7.62	4.66	0.00	175.68	0.00	112.81	12.59

[0026] Although chondroitin sulfate A and chondroitin sulfate C are contained in the protease processing object of fishes UROKO as shown in a table 2 thru/ or 4, chondroitin sulfate B is not contained.

[Translation done.]

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] The pattern in which the result of TLC analysis of the ethanol precipitate obtained in the example 1 and each preparation is shown.

[Translation done.]

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DRAWINGS

[Drawing 1]



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最終頁に続く

(54) 【発明の名称】 コンドロイチン硫酸類の製造方法

(57) 【要約】

【課題】 入手しやすい原料を用い、比較的に簡単な処理でコンドロイチン硫酸類を製造する。

【解決手段】 魚類のウロコを原料とし、これに可溶化処理、ポリペプチド除去処理及び分別処理を順次施す。

【特許請求の範囲】

【請求項1】 魚類のウロコを原料とし、これに可溶化処理、ポリペプチド除去処理及び分別処理を順次施すことを特徴とするコンドロイチン硫酸類の製造方法。

【請求項2】 水中で、魚類のウロコにプロテアーゼを作用させて、ウロコを可溶化し、次にこのようにして得られた可溶化液から副生ポリペプチドを除去したのち、分別沈殿によりコンドロイチン硫酸類を取得する請求項1記載のコンドロイチン硫酸類の製造方法。

【請求項3】 コンドロイチン硫酸類がコンドロイチン硫酸A及びコンドロイチン硫酸Cである請求項1ないし2のいずれかに記載のコンドロイチン硫酸類の製造方法。

【発明の詳細な説明】

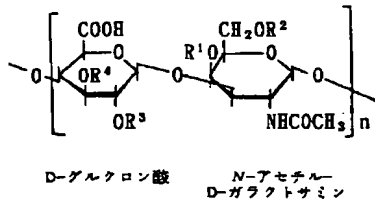
【0001】

【発明の属する技術分野】本発明は、魚類のウロコを原料として、点眼液や神経痛、関節痛の治療薬として有用なコンドロイチン硫酸類、特にコンドロイチン硫酸A及びコンドロイチン硫酸Cを製造する方法に関するものである。

【0002】

【従来の技術】コンドロイチン硫酸は、動物の軟骨組織や結合組織に広く分布している代表的なグリコサミノグリカンの1種であって、一般式

【化1】



で表わされる二糖を主要繰り返し単位40～100個が結合した構造を有し、硫酸基の数及び結合位置によって、コンドロイチン硫酸A（コンドロイチン-4-硫酸、 $R^1 = \text{SO}_3\text{H}$ 、 $R^2 \sim R^4 = \text{H}$ ）、コンドロイチン硫酸C（コンドロイチン-6-硫酸、 $R^2 = \text{SO}_3\text{H}$ 、 $R^1, R^3, R^4 = \text{H}$ ）、コンドロイチン硫酸D（ $R^2, R^3 = \text{SO}_3\text{H}$ 、 $R^1, R^4 = \text{H}$ ）、コンドロイチン硫酸E（ $R^1, R^2 = \text{SO}_3\text{H}$ 、 $R^3, R^4 = \text{H}$ ）、コンドロイチン硫酸K（ $R^1, R^4 = \text{SO}_3\text{H}$ 、 $R^2, R^3 = \text{H}$ ）、コンドロイチン硫酸B（デルマトン硫酸、D-グルクロン酸の多くが5-エピマー化されてL-イズロン酸となり、その R^1 が硫酸基になっている）に分けられている。

【0003】これらのコンドロイチン硫酸類の中のコンドロイチン硫酸Aは、主としてチョウザメの脊索、クジラの鼻軟骨を、またコンドロイチン硫酸Cは、主としてサメ軟骨をそれぞれ原料として製造されているが、原料の入手が困難な上に、煩雑な処理を必要とするため、大

量生産ができず、コスト高になるのを免れなかった。

【0004】

【発明が解決しようとする課題】本発明は、入手しやすい原料を用い、比較的簡単な処理でコンドロイチン硫酸類を製造することを目的としてなされたものである。

【0005】

【課題を解決するための手段】本発明者らは、コンドロイチン硫酸の製造原料について種々検討した結果、従来未利用のまま廃棄されていた魚類のウロコの中に、コンドロイチン硫酸A及びコンドロイチン硫酸Cが含まれ、これらは比較的容易に取り出すことができることを見出し、この知見に基づいて本発明をなすに至った。

【0006】すなわち、本発明は、魚類のウロコを原料とし、これに可溶化処理、ポリペプチド除去処理及び分別処理を順次施すことを特徴とするコンドロイチン硫酸類の製造方法を提供するものであり、この方法は、例えば水中で、魚類のウロコにプロテアーゼを作用させて、ウロコを可溶化し、次にこのようにして得られた可溶化液から副生ポリペプチドを除去したのち、分別沈殿によりコンドロイチン硫酸類を取得することからなっている。

【0007】

【発明の実施の形態】本発明方法において用いるウロコは、淡水魚、海水魚のいずれのウロコであってもよい。淡水魚のウロコとしては、例えばコイ、フナ、マス、キンギョなどのウロコを用いることができるし、海水魚のウロコとしては、例えばタイ、スズキ、サケ、ニシン、エツなどのウロコを用いることができる。これらのウロコは、単に水洗して汚れを除いたままで使用することができるが、必要ならば、あらかじめ120～130℃の温度で5～30分間加熱したものをを用いることもできる。

【0008】本発明方法における可溶化処理は、原料のウロコに酢酸カルシウムの希薄水溶液、好ましくはトリス塩酸で緩衝された水溶液を加え、ホモジナイズして懸濁液とし、これにプロテアーゼを加えて反応させることによって行われる。この反応は、通常30～40℃の温度において、5～40時間で完了する。この反応により、ウロコ中でコンドロイチン硫酸類と結合していたタンパク質部分が分離してポリペプチドを副生し、これはコンドロイチン硫酸類とともに水に溶解して水溶液になる。

【0009】このようにして得た水溶液は、次いで不溶分をろ過又は遠心分離により除去したのち、ポリペプチド除去処理に付する。これは、上記の不溶分を除いた水溶液をカチオン型イオン交換樹脂と接触させることにより行われる。この際、カチオン型イオン交換樹脂と接触するに先立って、必要に応じ水溶液を濃縮し、蒸留水に対して透析することもできる。

【0010】このようにして、コンドロイチン硫酸類を

含む水溶液が得られるが、この水溶液から目的生成物を分別するには、この水溶液中にエチルアルコールを加え、濃度を段階的に上げることによって、コンドロイチン硫酸類を沈殿させ、各画分ごとに捕集する。

【0011】このようにして、各画分から得られたコンドロイチン硫酸類は、信頼すべき由来の標品と対比させながら、消化酵素により加水分解し、生成する糖類を検出することにより同定された。この同定は、例えばコンドロイチナーゼを用いて各試料を加水分解し、得られた液を薄膜クロマトグラフィ（以下TLCと略す）により展開し、生成した不飽和二糖をジフェニルアミン試薬を用いて発色させ、同様に処理した標品のバンドと一致するか否かを確認することによって行われる。このようにして、本発明方法の生成物は、コンドロイチン硫酸A及びコンドロイチン硫酸Cであることが確認された。

【0012】

【実施例】次に実施例により本発明をさらに詳細に説明する。

【0013】実施例1

(1) ウロコの可溶性

コイウロコ60gをよく水洗したのち、120℃で20分間加熱処理した。次いで、0.02M-酢酸カルシウムを含む0.05M-トリス塩酸緩衝液(pH7.8)300mlに加熱処理したウロコとアクチナーゼE(科研製薬製)300mgを加えて、ホモジナイザーにより*

*均質化し、懸濁液を調製した。次にこの懸濁液を37℃で3日間反応させ、ウロコを完全に可溶化した。このようにして得た反応液を100℃で5分間加熱してアクチナーゼEを失活させ、40分間遠心分離して(20,000rpm)不溶成分を除去したのち、遠心上清を濃縮し、濃縮物を蒸留水に対して1夜透析した。透析処理した液を、Dowex50-x8(H⁺型)カラム(50×50mm)に通して上記の反応で遊離したポリペプチドを除去し、流出液を水酸化ナトリウムで中和したのち、凍結乾燥した。このようにして凍結乾燥物330mgを得た。

【0014】(2) 可溶化物の分別沈殿

(1)で得た凍結乾燥物330mgを0.5M-酢酸ナトリウム50mlに溶解したのち、酢酸によりpH4.5に調整し、この中にエタノールを注加し、エタノール濃度を段階的に上げることによってコンドロイチン硫酸を沈殿させ、エタノール濃度ごとの画分として捕集した。得られた各画分に1,000倍量(w/v)の2N-HClを加え、100℃において20時間加水分解したのち、DX-500糖分析装置(ダイオネクス社製)を用いてそれぞれの糖組成を分析した。その結果を表1に示す。

【0015】

【表1】

エタノール 画分(%)	沈殿物収量 (mg)	糖組成(μg/mg)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0~40	5.0	11.15	43.54	13.38	47.46	18.23	2.69	0.00
40~50	49.0	11.67	28.23	129.41	157.37	29.62	1.18	0.00
50~60	4.0	14.01	33.75	32.50	64.74	27.76	3.49	0.00
60~70	6.0	17.50	69.02	6.79	60.09	35.09	41.52	12.22
70~	70.0	3.43	20.47	0.00	7.33	13.49	39.53	15.63

【0016】なお、表中の糖の略号は以下の意味をもつ。

Fuc : フルクトース

Gal : ガラクトース

GlcUA : グルクロン酸

GalNAc : N-アセチルガラクトサミン

GlcNAc : N-アセチルグルコサミン

NeuAc : N-アセチルノイラミン酸

NeuGc : N-グリコリルノイラミン酸

【0017】この表から分るように、40~50%エタノール画分には他の画分よりも多量のグルクロン酸及びN-アセチルガラクトサミンが含まれ、またGlcUAとGalNAcとのモル比はほぼ1:1である。また、いずれの画分からもコンドロイチン硫酸Bの構成成分であるイズロン酸は検出されなかった。

【0018】(3) 同定試験

(2)で得た40~50%エタノール画分75μg及び

コンドロイチン硫酸A標品[生化学工業(株)製,SSGグレード,以下ChsAと略記する]とコンドロイチン硫酸C標品[生化学工業(株)製,SSGグレード,以下ChsCと略記する]の50μgずつを、それぞれ0.025M酢酸ナトリウム緩衝液(pH5.0)100μlに溶解し、コンドロイチナーゼ・アースロII[生化学工業(株)製]0.02単位を前記と同じ緩衝液2μlに溶かして加え、37℃で20時間反応させた。次いで反応液にエタノール300μlを加えて酵素を失活させ、遠心分離により不溶分を除去した。

【0019】得られた遠心上清を濃縮後、シリカゲル60TLCプレート(メルク社製)を用いて分析した。この結果を図1に示す。この際のTLCの展開液としては1-ブタノール/酢酸/蒸留水(容量2/1/1)を使用し、コンドロイチナーゼ・アースロIIの反応によって生成する不飽和二糖の発色にはジフェニルアミン試薬を用いた。この図1から分るように、40~50%エ

ノール画分から2個の不飽和二糖バンドが検出され、それらの移動度は標品として用いたChsA及びChsCから生成する2個の不飽和二糖のバンドとそれぞれ一致した。

【0020】実施例2

実施例1(1)におけるコイウロコ60gの代りにマダ*

*イウロコ142gを用い、実施例1と同様に処理して、エタノール沈殿物95mgを得た。このものの各エタノール画分ごとの糖組成を実施例1(2)と同様にして分析した。その結果を表2に示す。

【0021】

【表2】

エタノール 画分(%)	沈殿物収量 (mg)	糖組成(μg/mg)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0~40	6.1	5.30	167.44	26.53	181.04	29.13	23.07	0.00
40~50	23.3	2.40	102.91	197.65	264.73	83.68	11.25	7.85
50~60	11.7	0.44	53.58	25.50	57.11	33.75	58.51	55.34
60~70	5.2	4.10	4.77	6.66	93.67	0.00	99.41	29.32
70~	48.7	9.34	7.00	0.00	226.40	0.00	153.95	0.00

【0022】実施例3

実施例1(1)におけるコイウロコ60gの代りにエツウロコ26gを用い、実施例1と同様に処理して、エタノール沈殿物20.8mgを得た。このものの各エタノール※

※エール画分ごとの糖組成を実施例1(2)と同様にして分析した。その結果を表3に示す。

【0023】

【表3】

エタノール 画分(%)	沈殿物収量 (mg)	糖組成(μg/mg)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0~50	2.4	19.40	22.13	87.23	94.63	0.00	24.49	0.00
50~60	3.4	18.03	6.74	2.73	82.91	0.00	44.60	0.00
60~70	2.6	23.07	6.75	3.00	65.98	0.00	23.46	9.72
70~	12.4	12.93	8.40	1.55	138.72	0.00	113.39	25.76

【0024】実施例4

実施例1(1)におけるコイウロコ60gの代りにスズキウロコ23gを用い、実施例1と同様に処理して、エタノール沈殿物11.0mgを得た。このものの各エタノール★

★ノール画分ごとの糖組成を実施例1(2)と同様にして分析した。その結果を表4に示す。

【0025】

【表4】

エタノール 画分(%)	沈殿物収量 (mg)	糖組成(μg/mg)						
		Fuc	Gal	GlcUA	GalNAc	GlcNAc	NeuAc	NeuGc
0~50	1.4	4.91	11.01	67.92	82.36	0.00	11.57	0.00
50~60	1.7	1.93	131.13	2.63	14.25	0.00	15.03	0.00
60~70	1.2	6.19	8.14	0.00	27.09	0.00	18.95	0.00
70~	6.7	7.62	4.66	0.00	175.68	0.00	112.81	12.59

【0026】表2ないし4から分るように、魚類ウロコのプロテアーゼ処理物中には、コンドロイチン硫酸A及びコンドロイチン硫酸Cが含まれているが、コンドロイチン硫酸Bは含まれていない。

【0027】

【発明の効果】本発明によると、漁業廃棄物の1種であ

る魚類のウロコを原料として、医薬として利用価値の高いコンドロイチン硫酸A及びコンドロイチン硫酸Cを簡単な操作で製造することができる。

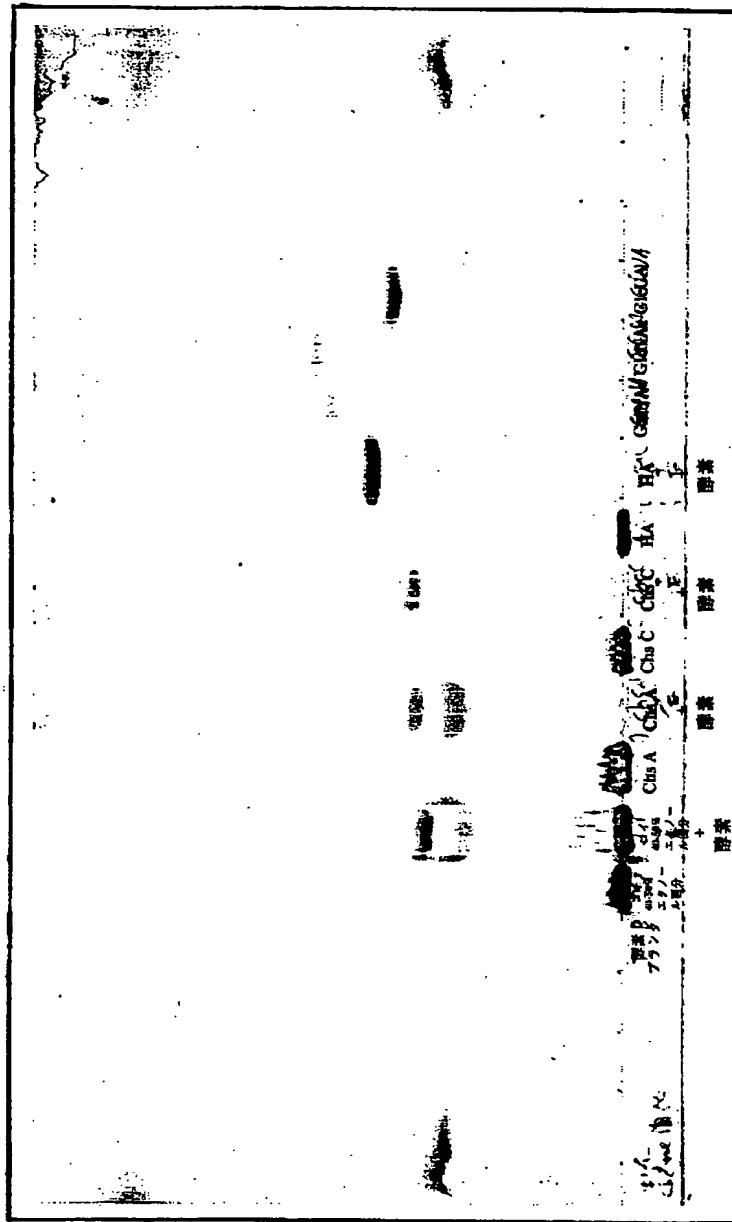
40 【図面の簡単な説明】

【図1】 実施例1で得たエタノール沈殿物と各標品のTLC分析の結果を示すパターン。

(5)

特開2001-139602

【図1】



【手続補正書】

【提出日】平成12年5月15日(2000. 5. 15)

【手続補正1】

【補正対象書類名】明細書

【補正対象項目名】0002

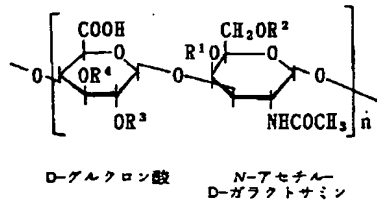
【補正方法】変更

【補正内容】

【0002】

【従来の技術】コンドロイチン硫酸は、動物の軟骨組織や結合組織に広く分布している代表的なグリコサミング

リカンの1種であって、一般式
【化1】



で表わされる二糖を主要繰り返し単位40～100個が結合した構造を有し、硫酸基の数及び結合位置によって、コンドロイチン硫酸A（コンドロイチン-4-硫酸、 $R^1 = \text{SO}_3\text{H}$ 、 $R^2 \sim R^4 = \text{H}$ ）、コンドロイチン硫酸C（コンドロイチン-6-硫酸、 $R^2 = \text{SO}_3\text{H}$ 、 $R^1, R^3, R^4 = \text{H}$ ）、コンドロイチン硫酸D（ $R^2, R^3 = \text{SO}_3\text{H}$ 、 $R^1, R^4 = \text{H}$ ）、コンドロイチン硫酸E（ $R^1, R^2 = \text{SO}_3\text{H}$ 、 $R^3, R^4 = \text{H}$ ）、コンドロイチン硫酸K（ $R^1, R^4 = \text{SO}_3\text{H}$ 、*

* $R^2, R^3 = \text{H}$ ）、コンドロイチン硫酸B（デルマタン硫酸、D-グルクロン酸の多くが5-エピマー化されてL-イズロン酸となり、その R^1 が硫酸基になっている）に分けられている。

【手続補正2】

【補正対象書類名】明細書

【補正対象項目名】0016

【補正方法】変更

【補正内容】

【0016】なお、表中の糖の略号は以下の意味をもつ。

Fuc : フコース
Gal : ガラクトース
GlcUA : グルクロン酸
GalNAc : N-アセチルガラクトサミン
GlcNAc : N-アセチルグルコサミン
NeuAc : N-アセチルノイラミン酸
NeuGc : N-グリコリルノイラミン酸

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